Methods xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Using magnets and magnetic beads to dissect signaling pathways activated by mechanical tension applied to cells

R.J. Marjoram [a,*], C. Guilluy [d,e], K. Burridge [a,b,c]

[a] Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC 27599-7295, United States
[b] Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599-7295, United States
[c] McAllister Heart Institute, University of North Carolina, Chapel Hill, NC 27599-7295, United States
[d] Inserm UMR_S1087, CNRS UMR_C6291, L'institut du Thorax, Nantes, France
[e] Université de Nantes, Nantes, France

ARTICLE INFO

Article history:
Received 2 May 2015
Received in revised form 21 August 2015
Accepted 27 September 2015
Available online xxxx

Keywords:
Mechanotransduction
Tension
VE-cadherin
Endothelial cell

ABSTRACT

Cellular tension has implications in normal biology and pathology. Membrane adhesion receptors serve as conduits for mechanotransduction that lead to cellular responses. Ligand-conjugated magnetic beads are a useful tool in the study of how cells sense and respond to tension. Here we detail methods for their use in applying tension to cells and strategies for analyzing the results. We demonstrate the methods by analyzing mechanotransduction through VE-cadherin on endothelial cells using both permanent magnets and magnetic tweezers.

1. Introduction

Analyses of physical forces applied to or produced within tissues and their molecular responses at the cellular level (i.e. mechanotransduction) have become important due to the role force-sensing has in normal biology and disease. For instance, endothelial cells of the vasculature experience shear and pulsatile forces under normal conditions as well as environmental stiffening upon progression of atherosclerosis. Cells detect these forces through cell–cell and cell–extracellular matrix (ECM) interactions, where cell-surface adhesive receptors form the links between neighboring cells or between cells and the ECM [1]. Adhesion receptors form the physical connection between the intracellular actin cytoskeleton and the surrounding environment, and some of these receptors sense tension differences and transduce this fluctuation into a chemical signal, such as activation of Rho GTPase signaling pathways [2]. How extracellular tension regulates cellular responses through cell–cell adhesion receptors is an important question in normal biology and disease.

Cadherins are a family of cell–cell adhesion receptors that are major components of adherens junctions and have been associated with tension sensing in cells [1]. Vascular endothelial-cadherin (VE-cadherin) is a classic cadherin mainly expressed on the plasma membrane of endothelial cells that line the luminal surface of blood vessels [3]. The initial discovery of VE-cadherin showed that this cadherin was involved with the barrier function of the endothelial layer by controlling permeability [4]. VE-cadherin at cell–cell junctions becomes disorganized during leukocyte trafficking between neighboring endothelial cells [5]. Tumor cells also induce disruption of the VE-cadherin contacts [6,7]. Under fluid shear stress, endothelial cells respond to the force through a mechanosensory complex involving VE-cadherin [8]. Under disease states, such as atherosclerosis, the physical environment of the endothelial cells changes and so does their response to external forces [9]. Coon and colleagues showed that the transmembrane domain of VE-cadherin serves an important role associating with VEGFR2/3 to form a mechanosensory complex in endothelial cells [10]. These data suggest that VE-cadherin has essential functions in mechanotransduction in endothelial cells to allow for responses to external forces from the extracellular environment.

Various tools have been utilized to study the effects of external forces on cells, including atomic force microscopy (AFM), optical tweezers, flow systems, PDMS microneedle substrates, and FRET tension sensors [11,12]. However, biochemical analysis is difficult with many of these techniques, whereas the use of magnetic beads to apply tension to a plate of cells readily facilitates biochemical assays. Magnetic beads also permit single cell assays, such as the measurement of bead displacements in response to repeated

* Corresponding author.
E-mail address: robin_marjoram@med.unc.edu (R.J. Marjoram).

http://dx.doi.org/10.1016/j.ymeth.2015.09.025
1046-2023/

pulses of applied force using magnetic tweezers. The ability to perform both single cell assays as well as bulk biochemical assays makes magnetic beads a valuable tool in the study of mechanotransduction, since this is not possible using approaches that can only analyze single cells such as optical tweezers or AFM. Another benefit is the ability to apply the force to a targeted receptor unlike more general force analysis tools (i.e. flow or traction force systems). Lastly, magnetic tweezers provide a much larger dynamic range of force application when compared to optical tweezers and AFM; they are able to apply weak forces (5 pN) similar to optical tweezers as well as strong forces (1 nN) on the same level as AFM [13]. In biology, cells are exposed to different types of force. Some forces are acute, some are sustained, some build slowly over time, and others are cyclical with periods of tension followed by relaxation. The different techniques all have advantages. The magnetic beads as used in assays by us mimic biological situations where force is applied relatively quickly for a short sustained period (permanent magnet) or a regimen of brief pulses of force with intervening periods of relaxation (magnetic tweezers). It should be noted that these force applications do differ slightly and can produce different cellular responses as was shown by the Fredberg group where they showed cellular reinforcement (stiffening) or fluidization (softening) was dependent on the force regime (frequency, amplitude) applied to the cell [14,15]. For these reasons, our lab utilizes magnetic beads for broad biochemical analyses using permanent magnets as well as fine tuned magnetic tweezers for pulling experiments to measure the stiffening response of cells to applied forces (Fig. 1).

Several different studies have used magnetic beads to explore how cells respond to mechanical forces exerted on cell adhesion molecules. In early work, Wang et al. used RGD-coated magnetic beads to apply a twisting force to integrins on the surface of endothelial cells and observed a stiffening response that was dependent on the actin cytoskeleton [16]. McCulloch's group used permanent magnets placed above cell cultures to pull vertically on collagen-coated magnetic beads adhering to the dorsal surface of cells. This allowed them to perform both single cell analysis, measuring for example increases in intracellular calcium in response to force, as well as bulk biochemical measurements on large populations of cells, such as analyzing protein tyrosine phosphorylation, which they showed increased in response to force [17,18]. In subsequent work the same group used this approach to show that sustained tension on integrins via magnetic beads coated with collagen activated RhoA [19]. Ingber and his colleagues used magnetic tweezers to examine the effects of applying tension on magnetic beads coated with integrin ligands and implicated RhoA signaling pathways in the cellular response [20]. Na et al. used the combination of FRET and magnetic twisting cytometry (MTC) to analyze rapid mechanochemical signaling in live cells and showed the prestressed cytoskeleton promoted rapid activation of Src upon force application [21,22]. Using this approach Poh et al. also showed that force application through integrins activated Rac1 and was independent of Src activity in human airway smooth muscle cells [23].

Following on from these studies, our lab has combined both biochemical analyses using permanent magnets with single cell experiments using magnetic tweezers to analyze the signaling pathways downstream from tension applied to integrins [24]. We used fibronectin-coated beads to pull on fibroblast integrins and showed the activation of RhoA was mediated by two distinct pathways that activate the Rho GEFs, LARG and GEF-H1. Additionally, activation of RhoA via these GEFs contributed to the observed cellular stiffening [24]. Magnetic beads and magnets have been used to apply force to other cell adhesion molecules. For example, Tzima's lab have shown that tension applied to PECAM-1, an endothelial cell adhesion molecule implicated in endothelial mechanotransduction, activates RhoA in an integrin-dependent pathway via GEF-H1 and LARG [25]. We showed that tension on ICAM-1 on endothelial cells causes cell stiffening and helps mediate transendothelial migration of leukocytes [26]. In another study, DeMali's lab used a similar approach to exert force on E-cadherin using magnetic beads coated with the extracellular domain of E-cadherin. They discovered that the tension-induced recruitment of vinculin depended on the phosphorylation of vinculin at Y822 [27]. Another study by Kim et al. used E-cadherin-coated magnetic beads to show α-catenin is an integral part of the force sensing apparatus at cell–cell junctions [28]. Other labs have also shown that vinculin, α-catenin, and actin are recruited to E-cadherin adhesions in response to force [29–31].

The versatility of using magnetic beads to generate tension is illustrated in a study in which the response of an organelle, the nucleus, to tension was examined. In this work, tension was applied to isolated nuclei using magnetic beads coated with antibodies against the nuclear envelope protein nesprin-1. Unexpectedly, successive applications of force resulted in

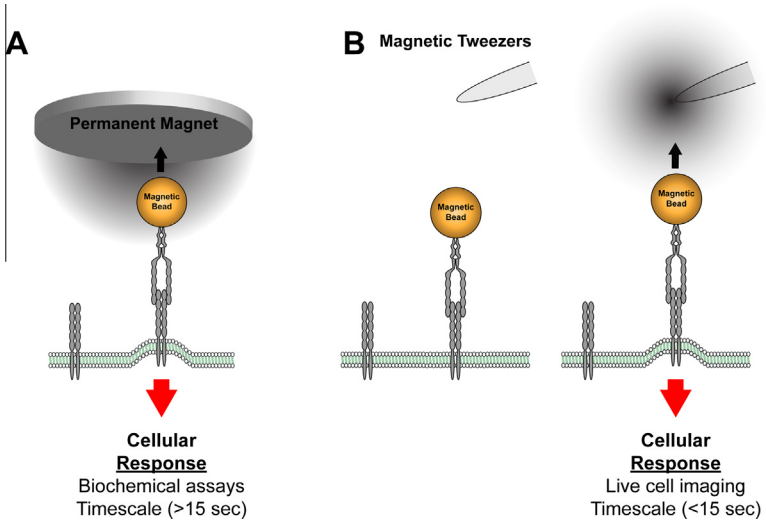

Fig. 1. Use of protein-coated magnetic beads in cell surface receptor tension experiments. (A) Permanent magnets can be used to apply pulling forces to ligand-coated magnetic beads adhered to cell surface receptors. Cell responses to tension on the timescale of 15 s and greater can be analyzed by various biochemical assays using this method. (B) Magnetic tweezers can utilize ligand-coated magnetic beads to probe tension responses by individual cells on shorter timescales than permanent magnets and measure real time responses using live cell imaging.

Please cite this article in press as: R.J. Marjoram et al., Methods (2015), http://dx.doi.org/10.1016/j.ymeth.2015.09.025